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AUTHOR(S):

HIKASA, YORINORI; MARUYAMA, IZUMI;
YOSHINAGA, MICHIO; HIRANO, MINORU; EGUCHI,
TAKASHI; SHIODA, RYUZO; TANIMURA, HIROSHI;
HASHIMOTO, KINYA

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Initiating Factors Of Gallstones, Especially Cholesterol Stones

by

YORINORI HIKASA, IZUMI MARUYAMA, MICHIO YOSHINAGA,
MINORU HIRANO, TAKASHI EGUCHI, RYUZO SHIODA,
HIROSHI TANIMURA and KINYA HASHIMOTO

From the Second Surgical Division, Kyoto University Medical School
(Director : Prof. Dr. CHUJI KIMURA)
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I. INTRODUCTION

Miscellaneous concepts on the initiating factors of gallstones have originated in an inflammatory theory advocated by NAUNYN and in a non-inflammatory bile stasis theory by ASCHOFF-BACMEISTER. LICHTWITZ, SCHADE et al. have tried to elucidate the initiating factors of gallstones with their colloidal theory. Many other investigators have declared the theories of which causes might be attributed to disturbances of metabolism, dysfunctions of the endocrine system, dysharmonies of the autonomic nervous system and abnormalities of constitution. Nowadays cholelithiasis is frequently encountered clinically but its etiology has not yet completely known.

We have already pointed out the possibility that the formation of cholesterol stones might be due to the metabolic disturbances in essential fatty acid (EFA), at the 6th Nutritional Meeting held in 1960 in our country²⁾. Getting a new powerful reinforcement of gas-liquid chromatography for chemical analysis, we have planned some experiments in order to verify the possibility. We have settled the mechanism in cholesterol stone formation in 1963 and published in 1964³⁾. Further investigations have led us to explain the detailed mechanism in cholesterol stone formation.

II. EXPERIMENTAL METHODS

Biochemical analyses were made with the liver-slices and aseptic bladder bile removed from the patients with gallstones and with gastric ptosis (control group) admitted at the Second Surgical Division, Kyoto University Hospital, without distinct disturbances of liver functions and infection of the bile, at surgical operations respectively. And at the same time, we have tried to produce gallstones experimentally, using rats, mice and hamsters.

We used the alkaline isomerization method originated by HOLMAN and HAYES¹⁾, and modified by JINDO in our clinic to determine the absolute amounts of fatty acids in the liver¹³⁾. Percentage of each fatty acid was determined by gas-liquid chromatographic separation with a SHIMADZU Sheisakusho Model GC-1-B instrument. Methylation of the extracted fatty acids was made with 2% sulfuric acid-methanol before gas-liquid chromatographic separation. Each component of the fatty acids separated by gas-liquid chromatography was identified, respectively, with the standard samples offered from National Institute for Health and Hormel Institute in United States of America⁵⁾. Esterified chole-

sterol contained in the liver and fatty acids composing lecithin excreted in the bile were separated by liquid-solid chromatography on silicic acid¹⁰⁾. The quantitative determination of cholesterol was made with ABELL's method¹¹⁾. Bile acids in the liver and bile were determined quantitatively with the BECKMAN DU quartz spectrophotometer according to the method described by MOSBACH¹⁶⁾. The gas-liquid chromatographic separations of the bile acids described in this publication were made with the GC-1-B instrument using a Nitril Silicon column¹⁹⁾.

III. RESULTS

In the patient's liver with gallstones, we have observed the following metabolic disturbances in EFA ; palmitoleic acid and oleic acid increase, 5, 8, 11-eicosatrienoic acid and 7, 10, 13-eicosatrienoic acid derived from the former two acids increase, 5, 8, 11, 14-eicosatetraenoic acid decreases in percentage, respectively, and thus the ratio of (20:3) acid to (20:4) acid is elevated. Cholesterol may also increase in the patient's liver in esterified as well as in total, whereas cholesterol may decrease when it combined with the fatty acids which had a role as an EFA in vivo and which activated cholesterol metabolism ; e. g. 5, 8, 11, 14-eicosatetraenoic acid, 7, 10, 13, 16-docosatetraenoic acid and 4, 7, 10, 13, 16-docosapentaenoic acid (Fig. 1). Bile acids, normal end-products of cholesterol, therefore, may be disturbed in their production and total bile acids are reduced in the bile. On the other hand, we have observed the decreased ratio of total bile acids to cholesterol and the increased ratio of dihydroxycholanolic acid to trihydroxycholanolic acid. Lecithin and its fatty acids constituents were reduced in their biliary excretion, but the fatty acids constituents were never changed in percentage.⁶⁾

We have easily produced the condition bearing a close resemblance to the changes in the fatty acids, bile acids and cholesterol in the liver and bile of the patients with gallstones as indicated at Figs. 2, 3, 4 by means of rearing rats on an EFA-deficient diet. Rats have no gall bladder, therefore, in accordance with their experimental results, we have observed the regular occurrence of gallstones with high cholesterol content in mice and hamsters of which composition of bile acids might be similar to human ones, when placed on an EFA-deficient diet for two months or three (Photos. 1, 2). Almost all the gallstones produced in the experimental animals were cholesterol stones, but some of them were pigment stones in which cholesterol content was very low.

It is noteworthy, however, that linoleic acid i. e. 9, 12-octadecadienoic acid was reduced always in amount in the liver of the experimental animals fed on an EFA-deficient diet, but on the other hand, this phenomenon was never observed in the patients with gallstones. Only at this point, therefore, the patterns of the data obtained from the experimental animals receiving an EFA-deficient diet don't coincide with those from the patients with gallstones. It is evident that the formation of human gallstones is not attributed to only a complete deficiency in EFA.

According to the experimental results, it is doubtless that the gallstones can be produced when happened to occur the following changes in the liver and bile ; diminution of cholesterol esterified with 5, 8, 11, 14-eicosatetraenoic acid, 7, 10, 13, 16-docosatetraenoic acid and 4, 7, 10, 13, 16-docosapentaenoic acid, especially with 5, 8, 11, 14-eicosatetraenoic

Fig. 1 Percentage of each Fatty Acid in the Liver of the Patients with Cholesterol Stones

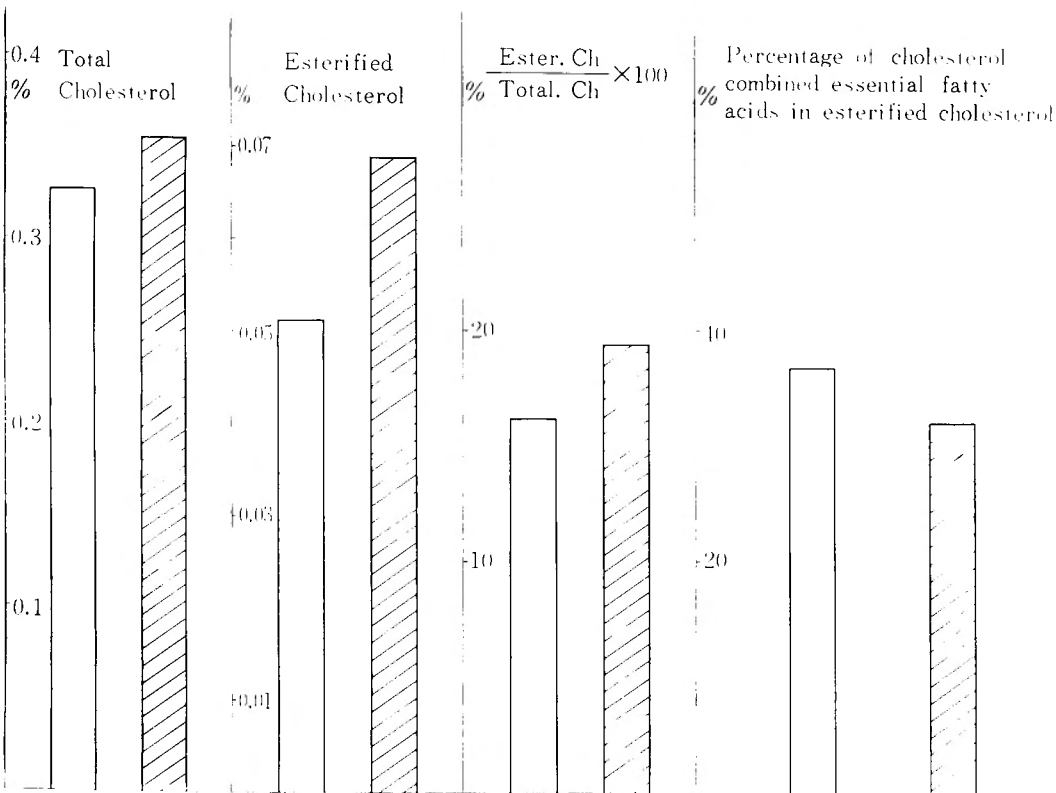
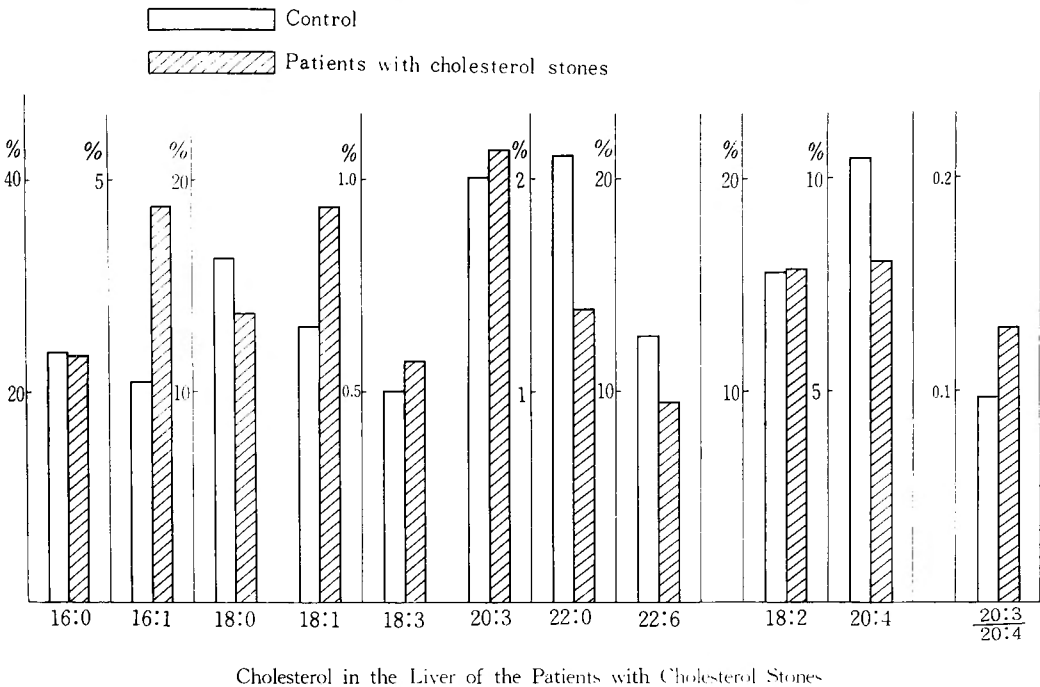
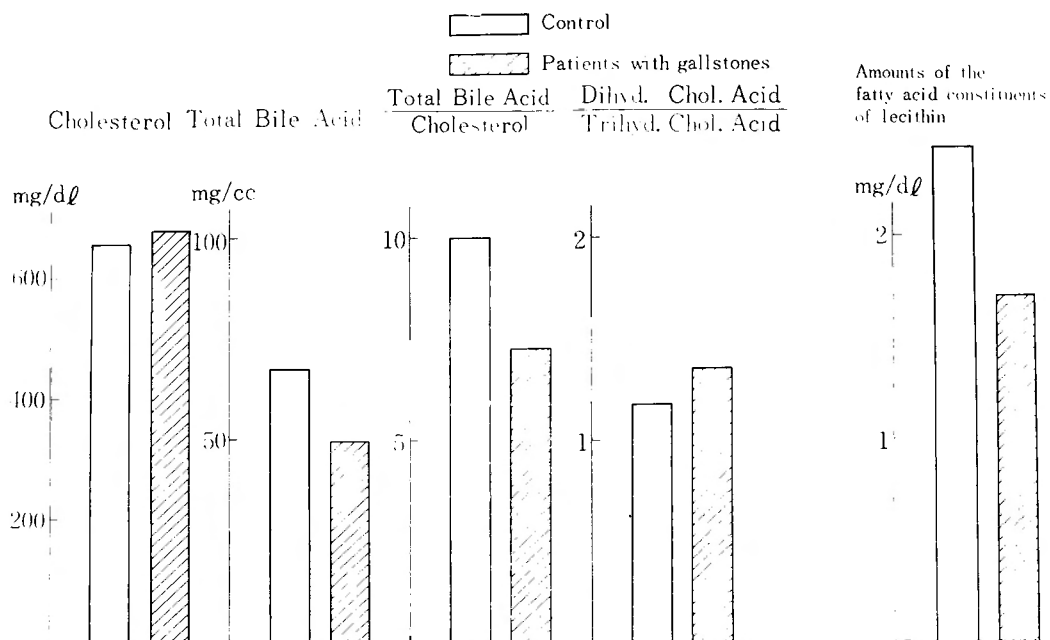


Fig. 2 Bile Components of the Patients with Gallstones



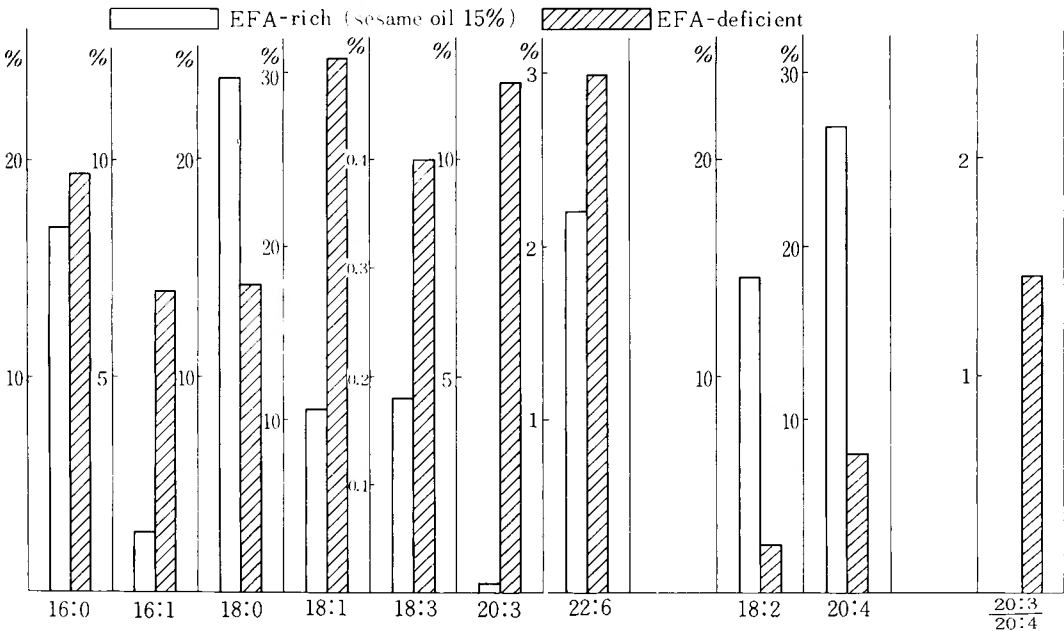
acid (arachidonic acid) to be participated in and activate its conversion in the liver, reduced production of bile acids, decreased total bile acids in the bile, the decreased ratio of total bile acids to cholesterol, the elevated ratio of dihydroxycholelanic acid to trihydroxycholelanic acid, the diminished biliary excretion of lecithin and its fatty acids constituents, decreased lecithin-bile salt complex, and lowered cholesterol-holding power.

In human body, EFA is mainly supplied with the vegetable oils containing excessive linoleic acid. If we could find out the conditions resulting in the patterns being peculiar to cholelithiasis in the experimental animals with sufficient administration of linoleic acid, those would be the true initiating factors in gallstone formation.

When a relative deficiency in pyridoxine is produced in the experimental animals receiving linoleic acid, the patterns of the fatty acids and bile acids in the liver and those of the bile acids in the bile coincide with those of the patients with gallstones. We have failed in observing an increase of cholesterol in total as well as in esterified in the liver and a diminution of lecithin in the bile (Figs. 5, 6).

When the experimental animals were fed on a linoleic acid-rich diet, under a relative deficient state of pyridoxine, and further animal fat containing inevitably cholesterol or fishes, shells and crustacea were added to the diet, the same patterns as those being characteristic of cholelithiasis can be obtained (Figs. 7, 8). They are also similar to the patterns observed in the animals receiving an EFA-deficient diet except only the changes of linoleic acid in the liver. Under the condition, we have succeeded in producing cholesterol stones at the rate of a high incidence in mice and hamsters (Photo. 3). The fact that human cholesterol stones contain much linoleic acid more than the other kinds of stones, especially bilirubin stones may suggest our concept being appropriate. When treated with

Fig. 3 Percentage of Each Fatty Acid in the Liver of Rats Fed on a EFA-rich Diet and a EFA-deficient Diet



Cholesterol and Bile Acid in the Liver of Rats Fed on a EFA-rich Diet and a EFA-deficient Diet

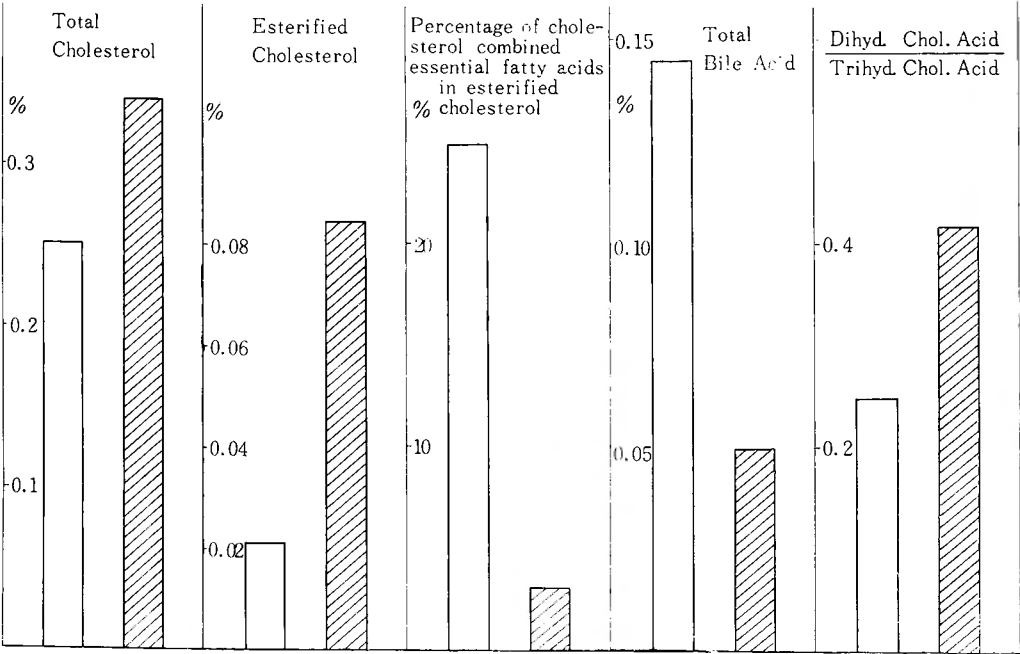
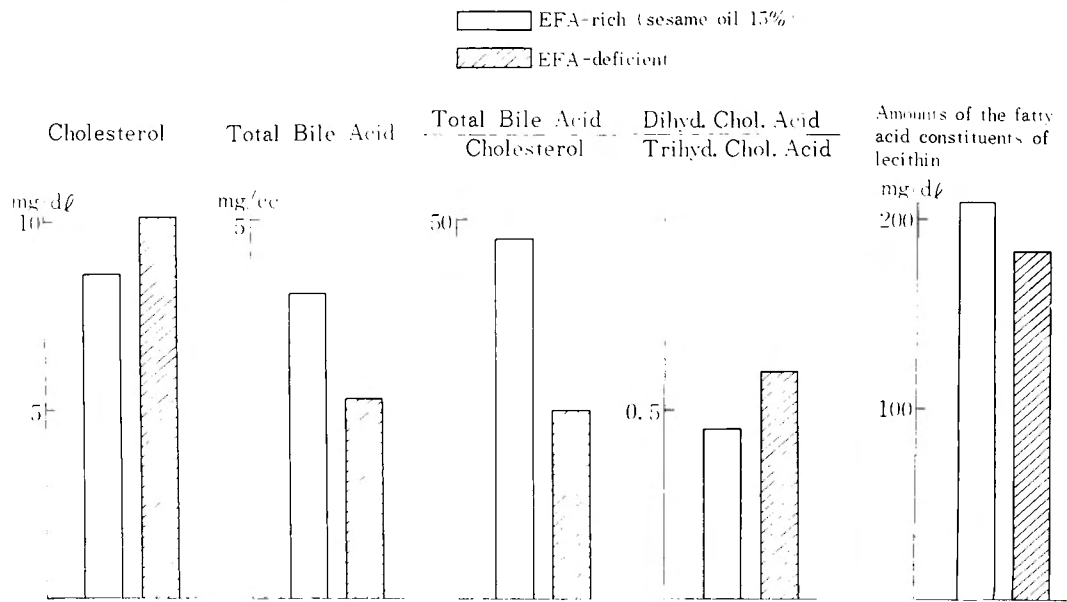
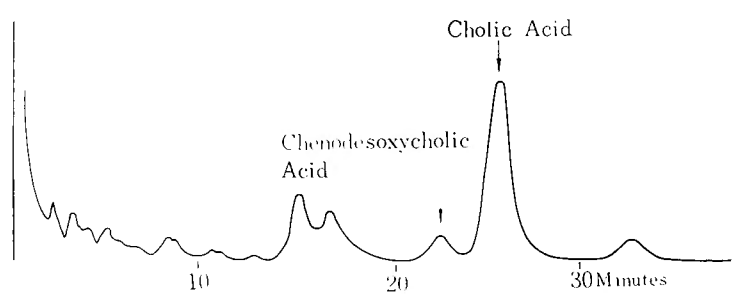


Fig. 4 Bile Components of Rats Fed on a EFA-rich Diet and a EFA-deficient Diet



Rats Fed on a EFA-rich Diet



Rats Fed on a EFA-deficient Diet

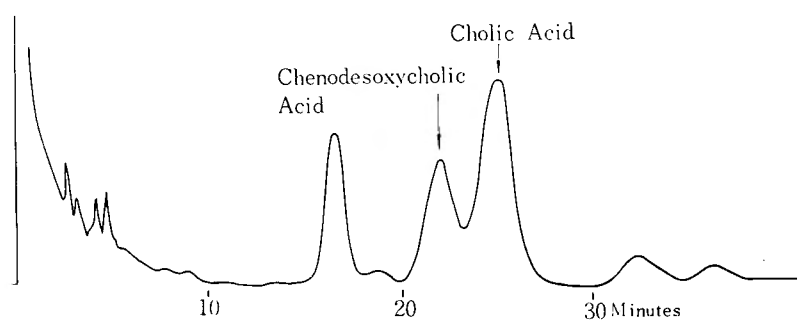
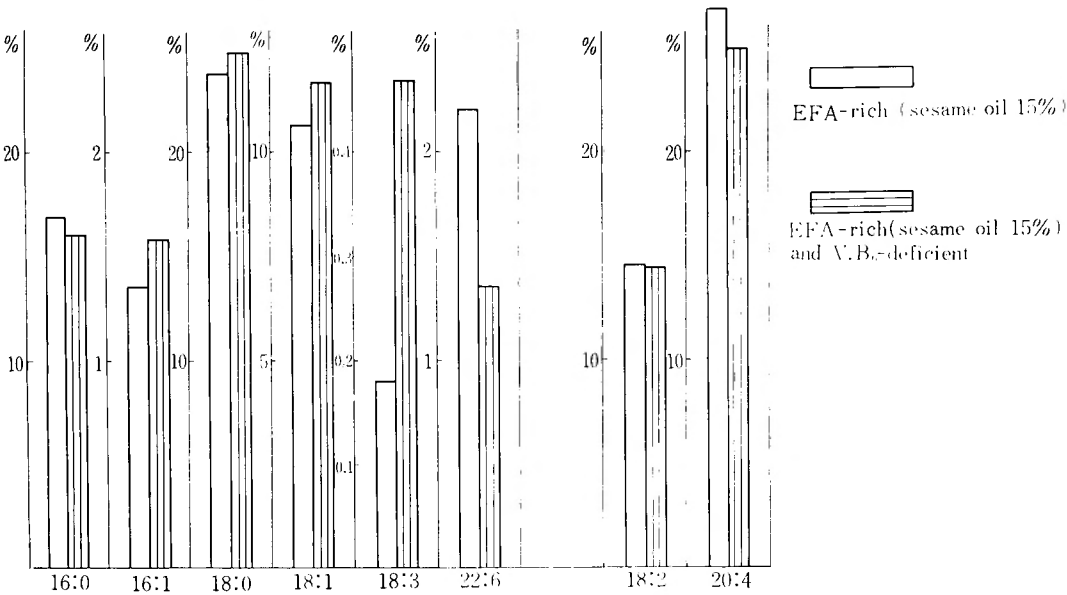


Fig. 5 Percentage of Each Fatty Acid in the Liver of Rats Fed on a EFA-rich Diet and a EFA-rich and V. B₆-deficient Diet



Cholesterol in the Liver of Rats Fed on a EFA-rich Diet and a EFA-rich and V. B₆-deficient Diet

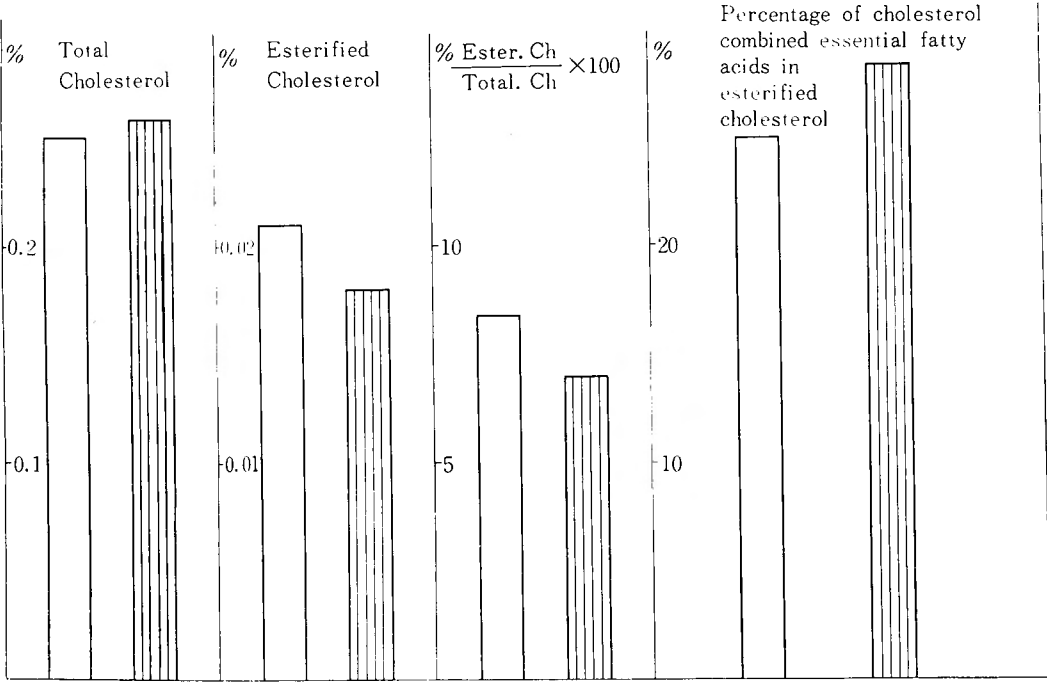
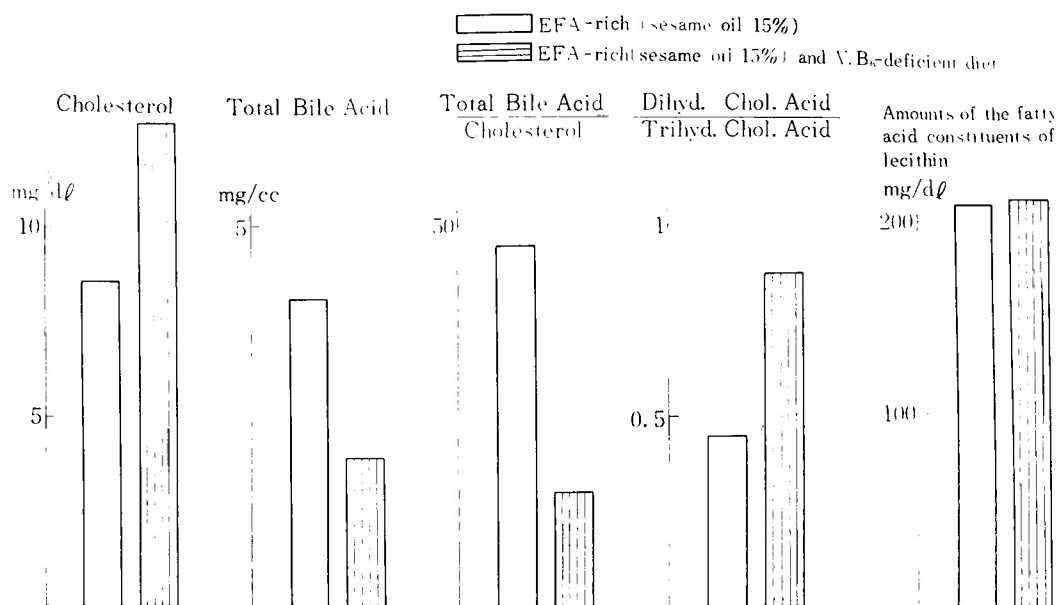


Fig. 6 Bile Components of Rats Fed on a EFA-rich Diet and a EFA-rich and V. B₆-deficient Diet

soya lecithin containing excessive linoleic acid and pyridoxine after a surgical drainage in the common bile duct of the patient with gallstones, the ratio of total bile acids to cholesterol in the drained bile shows a great increase as compared with a single administration of soya lecithin or non-treatment (Fig. 9).

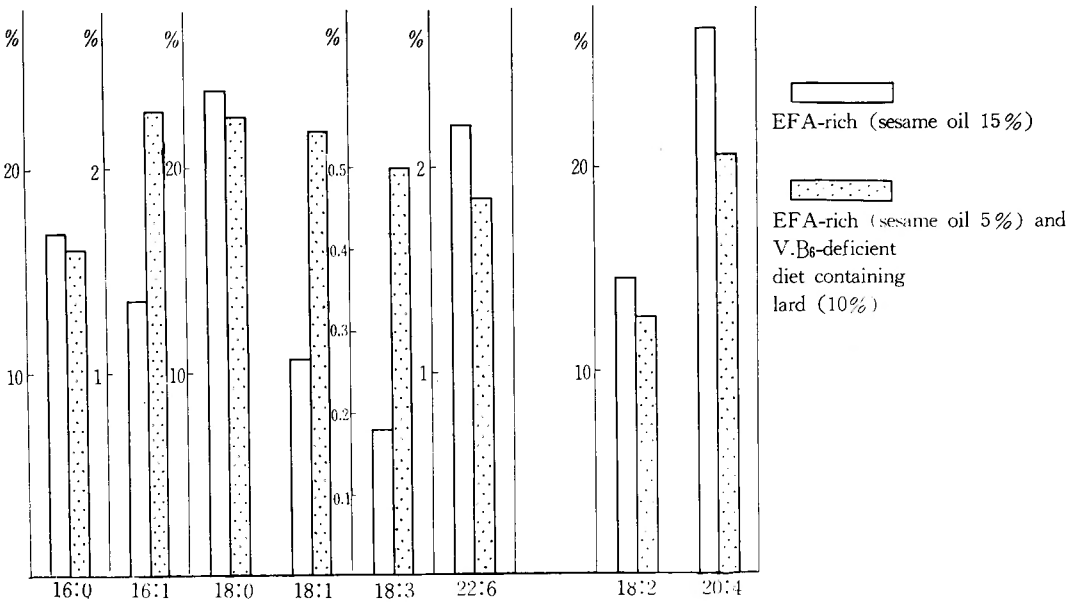
IV. DISCUSSION

As described at the introduction, the etiology of gallstone formation remains obscure. The mechanism in cholesterol stone formation may be attributed to the various factors as illustrated in the schema described by LARGE et al. in 1963.¹⁴⁾ It is generally accepted that the regional factors such as infection, inflammation, bile stasis and so on are responsible for cholesterol stone formation.

When the adrenocortical capacity of the cholelithiasis group was determined, it was remarkably reduced as compared with the control group. Tetraens in the former's serum were also decreased in its content.⁸⁾ On the other hand, when deficiency in EFA and its metabolic disturbances were produced in vivo experimentally, it is evident that the adrenocortical capacity was highly suppressed.⁷⁾ According to these facts, we have surmised for the first time in 1960 that the metabolic disturbances in EFA are important for gallstone formation.²⁾ So far as cholesterol stones are concerned, it is accepted that general and systemic factors such as the deficiency in EFA and its metabolic disturbances may become true factors in cholesterol stone formation.

When biochemical analyses were made with the liver and gall bladder bile of the patient with cholesterol stones, it was found the fact that the experimental results obtained from the patient were similar to those from the experimental animals fed on an EFA deficient-diet. Based on the fact, we have succeeded in producing gallstones experimentally

Fig. 7 Percentage of Each Fatty Acid in the Liver of Rats Fed on a EFA-rich Diet and a EFA-rich and V. B₆-deficient Diet Containing Lard



Cholesterol in the Liver of Rats Fed on a EFA-rich Diet and a EFA-rich and V. B₆-deficient Diet Containing Lard

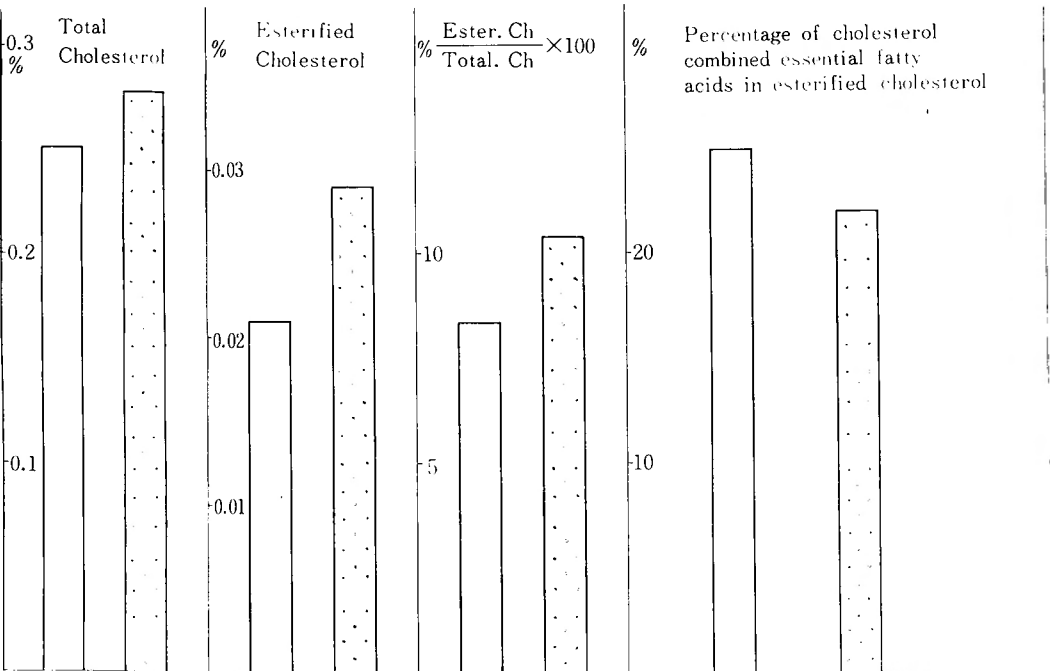


Fig. 8 Bile Components of Rats Fed on a EFA-rich Diet and a EFA-rich and V.B₆-deficient Diet Containing Lard

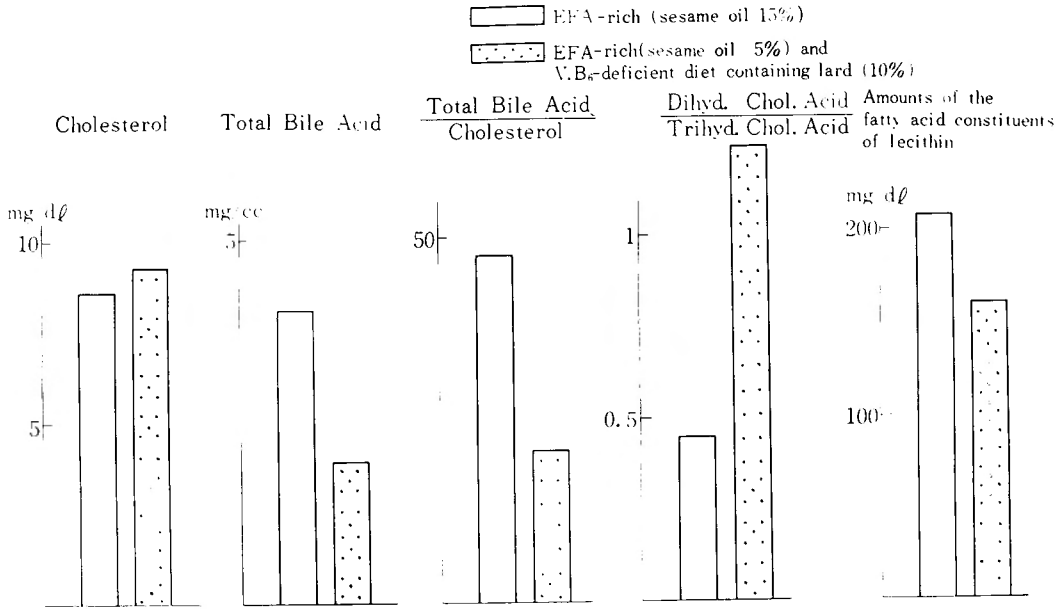
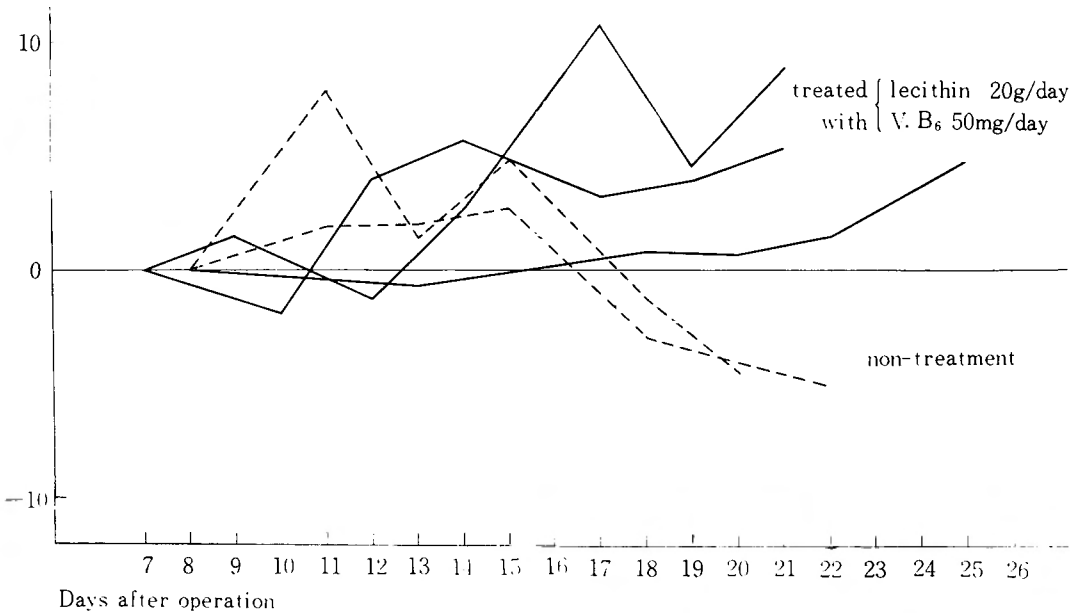


Fig. 9 The Changes in the Ratio of Total Bile Acids to Cholesterol in Bile, when Treated with Both Soya Lecithin and Pyridoxine after a Surgical Drainage in the Common Bile Duct of the Patient with Gallstones.



at the rate of a high occurrence by means of rearing mice and hamsters on an EFA deficient-diet for two months or three.

Fatty acids in the liver which combine with cholesterol to activate its metabolic degradation and at the same time play the part of an EFA *in vivo*, for example, 5, 8, 11, 14 -eicosatetraenoic acid, 7, 10, 13, 16-docosatetraenoic acid and 4, 7, 10, 13, 16-docosapentaenoic acid are extremely reduced quantitatively, palmitoleic acid and oleic acid synthesized *in vivo* increase as if they compensate for want of EFA. Cholesterol in esterified as well as in total increase in the liver. Total bile acids are reduced in the bile. Lecithin may also decrease to lower lecithin-bile salt complex (LBSC) in the bile.⁶⁾ The ratio of total bile acids to cholesterol is lowered to weaken cholesterol-holding power. The ratio of dihydroxycholanolic acid to trihydroxycholanolic acid is elevated.¹²⁾ When such coherent changes happen to occur simultaneously *in vivo*, gallstones can be initiated.

Cholesterol stone formation, however, is not due to only the deficiency in EFA, because the data obtained from the experimental animal's liver and bile receiving a diet without EFA are bearing a rough resemblance to those from the patients with cholesterol stones and although 9, 12-octadecadienoic acid i. e. linoleic acid is reduced in percentage as well as in absolute amount in the former, it is not reduced or if reduced, it is reduced so slightly its diminution cannot be proved in the latter. In other words, as the patient with gallstones intake linoleic acid sufficiently from the vegetable oils and the other food stuffs, the deficiency in linoleic acid does not occur. It is indicated clearly by the data that the metabolic disturbances in EFA such as 9, 12-octadecadienoic acid, 5, 8, 11, 14-eicosatetraenoic acid, 7, 10, 13, 16-docosatetraenoic acid and 4, 7, 10, 13, 16-docosapentaenoic acid are not induced through the deficiency but through the disturbed utilization in EFA.

How can be the metabolic disturbances in EFA produced? Deficiency in pyridoxine is thought in the first place.

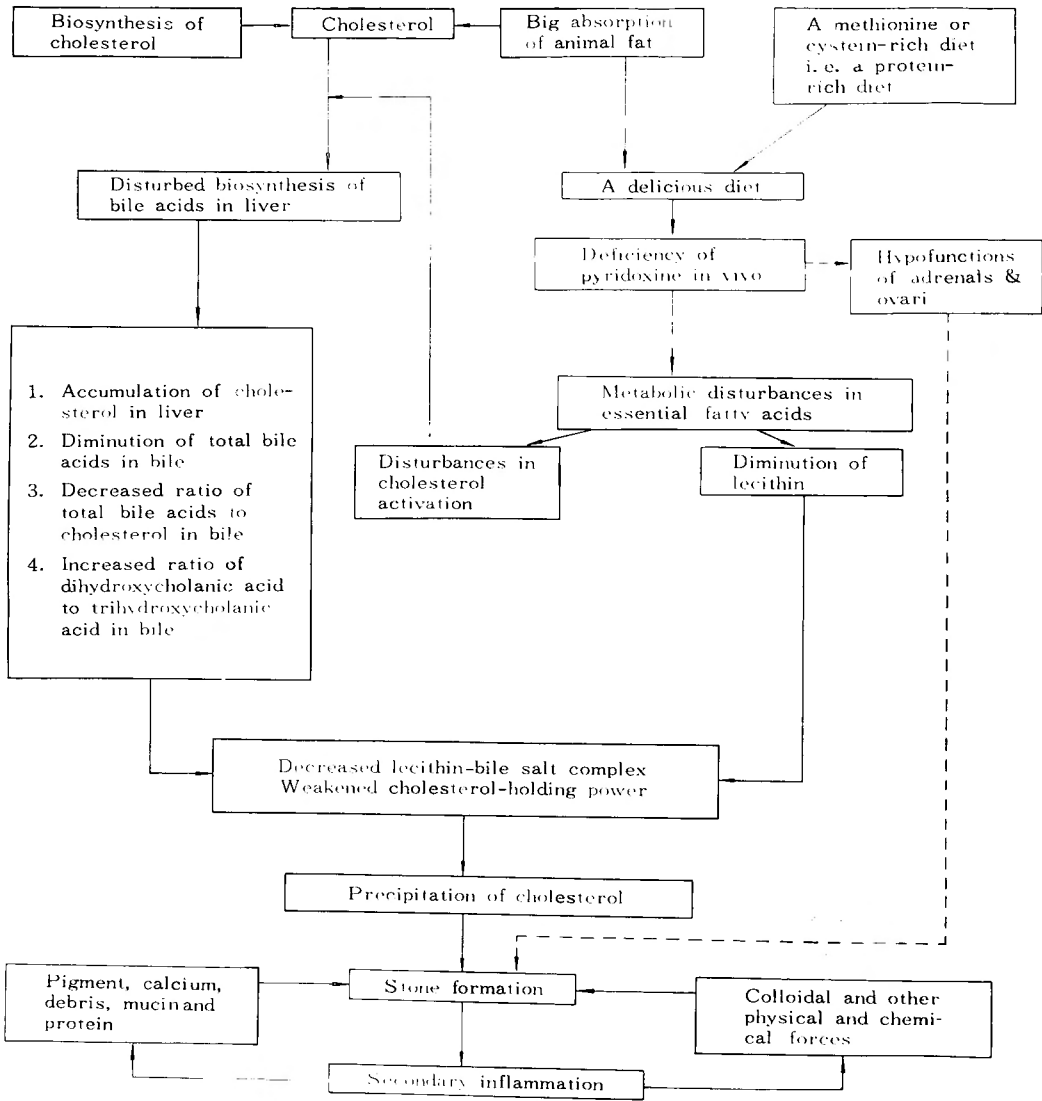
Using another group of the animals fed on a diet containing enough linoleic acid under a relative deficient state in pyridoxine, we have examined the changes of cholesterol and fatty acids in the liver and bile and further bile acids in the liver and bile. The changes of the fatty acids and bile acids in the liver and bile are very similar to those of the patient with gallstones, but in this case we cannot observe the elevation of both total cholesterol and esterified cholesterol in the liver and the diminution of lecithin in the bile. When animal fat which was composed of chiefly several kinds of saturated fatty acids, oleic acid and a little EFA was added to the diet, the changes in the liver and bile coincide exactly with those of the patients with gallstones and the regular occurrence of cholesterol stones has been observed in the experimental animals.

A large absorption of saturated fatty acids or amino acids, especially methionine and cysteine may result in the deficiency in pyridoxine. Deficiency in pyridoxine and increased cholesterol supplement are brought about simultaneously *in vivo* in those who are used to take ample proteins and saturated fatty acids i. e. a rich diet¹⁷⁾¹⁸⁾. As a matter of fact, it is informed that the gallstones composed chiefly of cholesterol occur frequently in European and American taking animal fat in a large quantity more than Japanese whose gallstones are consisted mainly of bilirubin-pigment. Recently the frequent occurrence of cholesterol stones has been observed in the city dwellers who are apt to take a rich diet

with the development of life and the large consumption of animal fat in our country.

When once gallstones are initiated in the gall bladder, they may invite a secondary inflammation in the gall bladder and bile ducts. The inflammation therefore, changes pH of bile and increases the secretion of mucin-like substances and abnormal proteins in the bile. It is thought that the various changes produced by the inflammation may damage the colloidal stability of the bile and may result in the initiation and development of

Fig. 10 Our Concept of the Total Process of Cholesterol Stone Formation



gallstones¹³⁾. If only cholesterol contained in mixed or combined gallstones begin to dissolve according to the changes in bile components after their initiation, it is possible that bilirubin-pigment stones may be produced. It is impressed by the fact that some of bilirubin-pigment stones in man were initiated by such a mechanism.

As EFA is also included in some organs secreting steroid hormones, for example, adrenals and ovari, hypofunctions in these organs may occur when the metabolism of EFA is disturbed *in vivo*.⁷⁾ It is not neglected that the functions of the endocrine system may be concerned with the initiation of gallstones. It is indicated by the fact that cholesterol stones occur frequently in women more than in men.

Our concept of the total process of cholesterol stone formation in man is illustrated in Fig. 10. We understand that cholesterol stone formation is attributed to the systemic initiating factors.

We have reported for the first time in 1960 about the possibility that the gallstones, especially cholesterol stones might be due to the deficiency in EFA or its metabolic disturbances. We have never changed our concept. We have published the experimental results on the stone formation repeatedly.⁹⁾ MIYAKE, a chief of the Surgical Department of Kyushu University Medical School has agreed with our concept on the initiating factors of cholesterol stones at the 64th General Meeting of Japanese Surgical Society. Cholesterol stones in man are not attributed to the deficiency in EFA, as we have already pointed out. The metabolic disturbances in EFA should be consistently responsible for cholesterol stone formation.

V. SUMMARY

We have discussed on the initiating factors of cholesterol stones clinically as well as experimentally. It is evident that they are attributed to the systemic factors i. e. the metabolic disturbances in EFA and that the cholesterol stones occur frequently in those who are used to take a delicious diet containing excessive animal fat and protein.

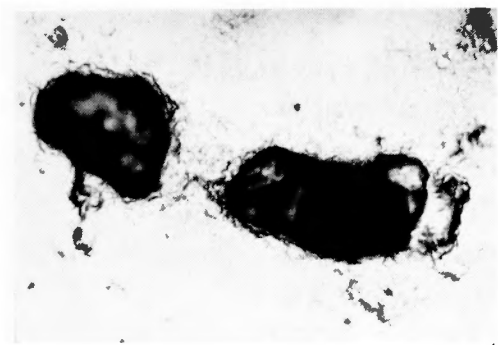
Regional factors such as ascending, descending and hematogenous infections of the bile have been accepted for long time to be responsible for cholesterol stone formation. They are not primary. They are invited secondarily by the infections when once gallstones are initiated. They are not true initiating factors in cholesterol stone formation.

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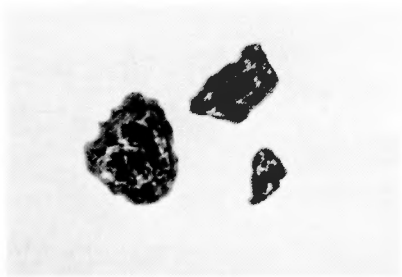
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Photo 1



Gallstones in mouse

Photo 2



Gallstones in Hamster

Photo 3



Gallstones in Hamster

和文抄録

胆石，特にコレステロール系結石の成因に
関する実験的並びに臨床的研究

京都大学医学部外科学教室第二講座（指導：木村忠司教授）

日笠頼則・丸山 泉・吉永道生・平野 穰
江口 隆・塩田隆三・谷村 弘・橋本欣也

胆石症が日常の臨床に於て屢々認められる疾患であるにも拘らず，その成因は依然として不明の状態にあるものといつてよい。

われわれは夙に1960年に行われた第6回栄養談話会席上順天堂大学福田 保教授の「コレステロール系結石の成因を如何に考えているか」との御質問に対して，われわれは「コレステロール系結石は不可欠脂酸の代謝障碍によつて形成されるに至る」可能性のあることをはつきり御指摘申上げると共にわれわれの考え方が何処にあるかをはつきりと御返答申上げたのである。

というのも，当時既にわれわれは不可欠脂酸が肝，副腎，心筋等に特に多量に含有されている事実を野田・平山の Paper chromatography あるいは教室神藤の改良した Alkaline isomerizing methode を以て確証すると共に，他方不可欠脂酸の欠乏した個体に於ては，その Adrenocortical capacity が著しく減弱するもので，副腎に於けるコレステロール代謝はそこに同時に共存する

不可欠脂酸のそれと密接不可分の關係にあり，要するに，個体の副腎皮質機能の如何は副腎中のコレステロール量の多寡によつて左右されるのではなくして，それとエステル結合する相手の脂酸の如何によつて規定され得る事実を明らかにして居り，当然 Glucocorticoids の Precursor であるコレステロールがまた胆汁酸の Precursor でもある事實に鑑み，analogisch な關係が必ずそこに成立するものと考えたからに他ならない。

その後，Gas-liquid chromatography 装置の入手と共に，以上のようなわれわれの考案が果たして当を得たものであるかどうかについての実験的並びに臨床的研究を開始，その後数次に亘りこの方面の研究成績を発表，コレステロール系結石の成因は結局第10図の如くに要約し得ることを明らかならしめることが出来たのである。而して，その成因としては，京都大学医学部故松尾 巖教授の夙に提唱されたように，全身的要因がその真の成因と解すべきである。

訂正

Errors :

(Amendment)

1) To be omitted the sentence i. e. "Miyake,.....Society.," printed from 16 line to 18 at 613, No. 3, Vol. 33, Archiv für Japanische Chirurgie.

2) To correct the Fig. 10 at 612, No. 3, Vol. 33, Archin für Japanische Chirurgie to the new one printed hereunder.

